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The primary structure of two polypeptide chains from preparations of homeostatic thymus hormone (HTH_α and HTH_β)

Entire-chain identities to two histones

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The primary structures of the 2 polypeptide chains (HTH_α and HTH_β) of the homeostatic thymus hormone (HTH) were determined. The entire structures were found to be identical to those of histones H2A and H2B, respectively, without evidence for sub-types, proteolytic processings, or other peptide fragments. The results show that suggestions for new extranuclear and hormone-like histone functions apply to HTH preparations with intact protein chains of the H2 histones

Thymus hormone *Histone* *Homology* *Amino acid sequence analysis*

1. INTRODUCTION

Endocrine activities of the thymus are well established, as well as thymic functions in proliferation and differentiation of lymphoid cells. One of the thymus hormones, for which additional interrelationships with endocrine glands have been shown, is the homeostatic thymus hormone, HTH, identified by Bezssonoff and Comsa [1] and further purified by Bernardi and Comsa [2]. It appears to antagonize the influence of several hormones (thyroxine, ACTH, desoxycorticosterone, TSH, and gonadotropins) but to be synergistic with one (growth hormone) [3]. HTH further suppresses consequences of thymectomy [4], and an HTH-like activity has been reported to be present in lymph nodes, spleen, and urine, disappearing after thymectomy [5].

Purification of HTH was recently shown to yield 2 polypeptide chains, HTH_α and HTH_β, apparently corresponding to histones H2A and H2B [6]. Since extracellular or hormone-like activities of histones are otherwise unknown, a determination of the primary structure of both HTH components was necessary to confirm the identity with the histones H2A and H2B. Especially, since histones can be chemically modified, since their dibasic residues may be important for cleavages of hormonal pro-forms, and since different homologies have been suggested [7], the entire HTH structure had to be established. Results of such HTH_α and HTH_β determinations are now reported, showing that the primary structures are identical with those of histones H2A and H2B, respectively. No evidence for the presence of processed or chemically modified forms was obtained. The results strongly support the conclusions that histones may have previously unknown hormonal functions, thereby revealing a novel type of interaction.

2. MATERIALS AND METHODS

2.1. *Purification*

HTH was isolated from calf thymus as described

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[1,2] and its 2 components, HTH_α and HTH_β, were further purified by high-performance liquid chromatography (HPLC) [6].

2.2. *Amino acid sequence determination*

HTH_α and HTH_β were cleaved with CNBr (100 μg/ml) in 70% formic acid for 24 h at room temperature, and with proteolytic enzymes in 0.1 M ammonium bicarbonate, as described for other protein studies [8]. CNBr fragments obtained were pre-fractionated by chromatography on Sephadex G-50 and were finally purified by reverse-phase HPLC in purification schemes suitable for protein analyses [8]. Enzymatic peptides were purified directly by reverse-phase HPLC. Pure peptides were analyzed for total compositions with a Beckman 121M amino acid analyzer after acid hydrolysis in evacuated tubes for 24 h at 110°C in 6 M HCl/0.5% phenol. Amino acid sequences were determined manually utilizing the dimethylaminoazobenzene isothiocyanate method [9] monitored for by-products [10], as well as by liquid phase sequencer analysis in a Beckman 890D instrument, utilizing a 0.1 M Quadrol peptide program and application in glycine-pretreated polybrene [11]. Phenylthiohydantoins were identified by reverse-phase HPLC [12], supplemented where necessary by thin-layer chromatography [13].

Table 1

Total compositions of intact HTH_α and HTH_β

Amino acid	HTH_α	HTH_β
Asx	7.6 (7)	7.4 (6)
Thr	5.3 (5)	7.7 (8)
Ser	4.5 (4)	13.7 (14)
Glx	11.8 (12)	11.4 (10)
Pro	4.4 (5)	7.2 (6)
Gly	13.2 (14)	9.9 (7)
Ala	15.9 (17)	13.0 (13)
Val	8.4 (8)	8.8 (9)
Met	– –	2.2 (2)
Ile	4.6 (6)	6.4 (6)
Leu	13.9 (16)	6.9 (6)
Tyr	2.7 (3)	5.1 (5)
Phe	0.9 (1)	2.4 (2)
Lys	14.4 (14)	19.9 (20)
His	4.2 (4)	2.9 (3)
Arg	13.7 (13)	7.4 (8)

Values shown are molar ratios after hydrolysis for 24 h and without corrections for impurities, slow release or hydrolytic destruction. Values within parentheses show results from sequence analyses

3. RESULTS

The results of the primary structure determination are shown in fig.1. Direct liquid phase sequencer analysis of intact HTH_β yielded the structure of the first 40 residues, whereas the N-terminally blocked HTH_α could not be directly submitted to sequential degradations. Fragmentation of the HTH_α : HTH_β mixture with CNBr yielded 6 fragments: uncleaved HTH_α (since this protein chain lacks Met), and 5 peptide fragments of HTH_β. The latter were derived from non-stoichiometric cleavages at the 2 methionine residues of the protein chain and therefore formed

Table 2

Total compositions of all CNBr fragments of HTH_β

Amino acid	CB1	CB1a	CB2	CB3	CB3a
Cys	– –	– –	– –	– –	– –
Asx	2.2 (2)	2.6 (2)	– –	3.8 (4)	3.7 (4)
Thr	1.9 (2)	2.2 (2)	– –	6.1 (6)	6.0 (6)
Ser	6.3 (7)	6.6 (7)	– –	6.4 (7)	6.2 (7)
Glx	4.2 (4)	4.2 (4)	– –	6.2 (6)	6.3 (6)
Pro	5.1 (5)	4.5 (5)	– –	1.1 (1)	1.1 (1)
Gly	3.3 (3)	4.1 (4)	1.0 (1)	3.2 (3)	4.3 (4)
Ala	6.1 (6)	6.2 (6)	– –	7.4 (7)	7.1 (7)
Val	4.9 (5)	5.4 (5)	– –	3.8 (4)	3.9 (4)
Met	0.3 (1)	0.5 (2)	0.3 (1)	– –	0.3 (1)
Ile	1.0 (1)	1.9 (2)	1.0 (1)	3.9 (4)	4.7 (5)
Leu	1.0 (1)	1.1 (1)	– –	5.1 (5)	4.9 (5)
Tyr	2.8 (3)	3.2 (3)	– –	2.0 (2)	1.9 (2)
Phe	– –	– –	– –	2.0 (2)	1.9 (2)
Lys	14.8 (15)	14.7 (15)	– –	5.1 (5)	5.2 (5)
His	1.0 (1)	1.0 (1)	– –	2.1 (2)	1.8 (2)
Arg	2.0 (3)	2.6 (3)	– –	4.0 (5)	4.2 (5)

Stoichiometric cleavages at 2 methionine residues yield 3 CNBr fragments, the N-terminal CB1, the middle 3-residue CB2, and the C-terminal CB3 (cf. fig.1). However, because of non-stoichiometric cleavages at both methionine residues, CNBr fragments CB1a and CB3a were also obtained, corresponding to the terminal fragments (CB1 and CB3) elongated by the 3-residue middle fragment (CB2, cf. fig.1)

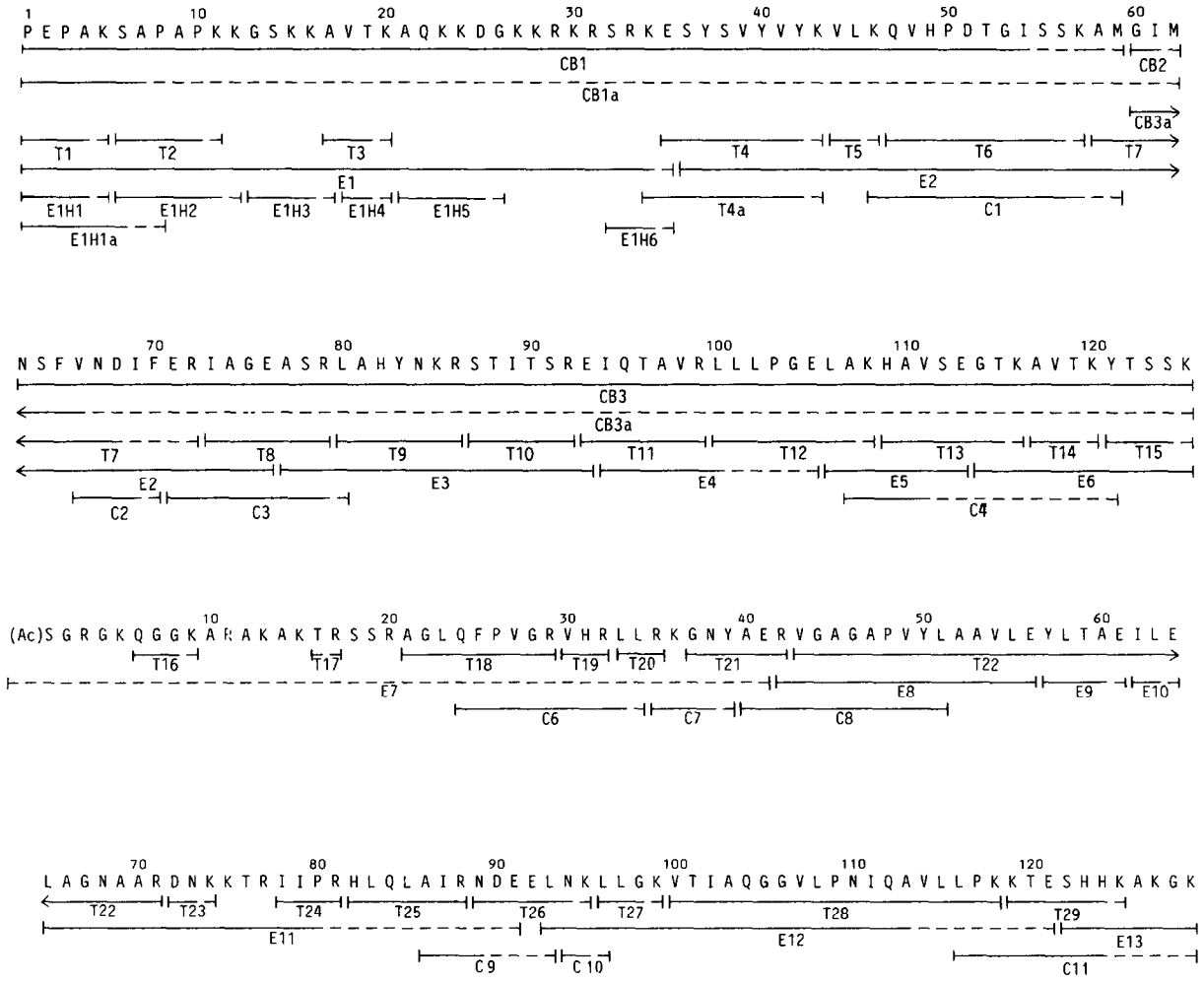

Fig.1. Results of sequence analysis of HTH_β (top) and HTH_α (bottom). All tryptic peptides (T) analyzed, as well as peptides obtained by digestions with staphylococcal Glu-specific protease (E), chymotrypsin (C), thermolysin (H; redigestion of E1, single unconventional cleavages), and cyanogen bromide (CB), are shown by solid lines for regions passed by Edman degradations and by dashed lines for regions proven by total compositions only. Peptides are numbered consecutively from the N-terminus, starting with HTH_β.

partly overlapping structures. All fragments analyzed are shown in fig.1, and the non-stoichiometric cleavages immediately overlapped the CNBr fragments. The supporting total compositions are given in table 2.

The purification of enzymatic peptides obtained by digestion with trypsin and staphylococcal Glu-specific protease is shown in fig.2. The compositions of all these peptides (not shown) are also in full agreement with the structures deduced. Except for some of the residues in the N-terminally blocked region of HTH_α, all positions in both HTH_α and HTH_β were completely checked.

4. DISCUSSION

All structures analyzed of HTH_α and HTH_β showed complete agreement with the known structures (from calf and man) of histones H2A and H2B, respectively. No inconsistencies with known histone structures were detected, neither was evidence for sub-types of H2A and H2B found,

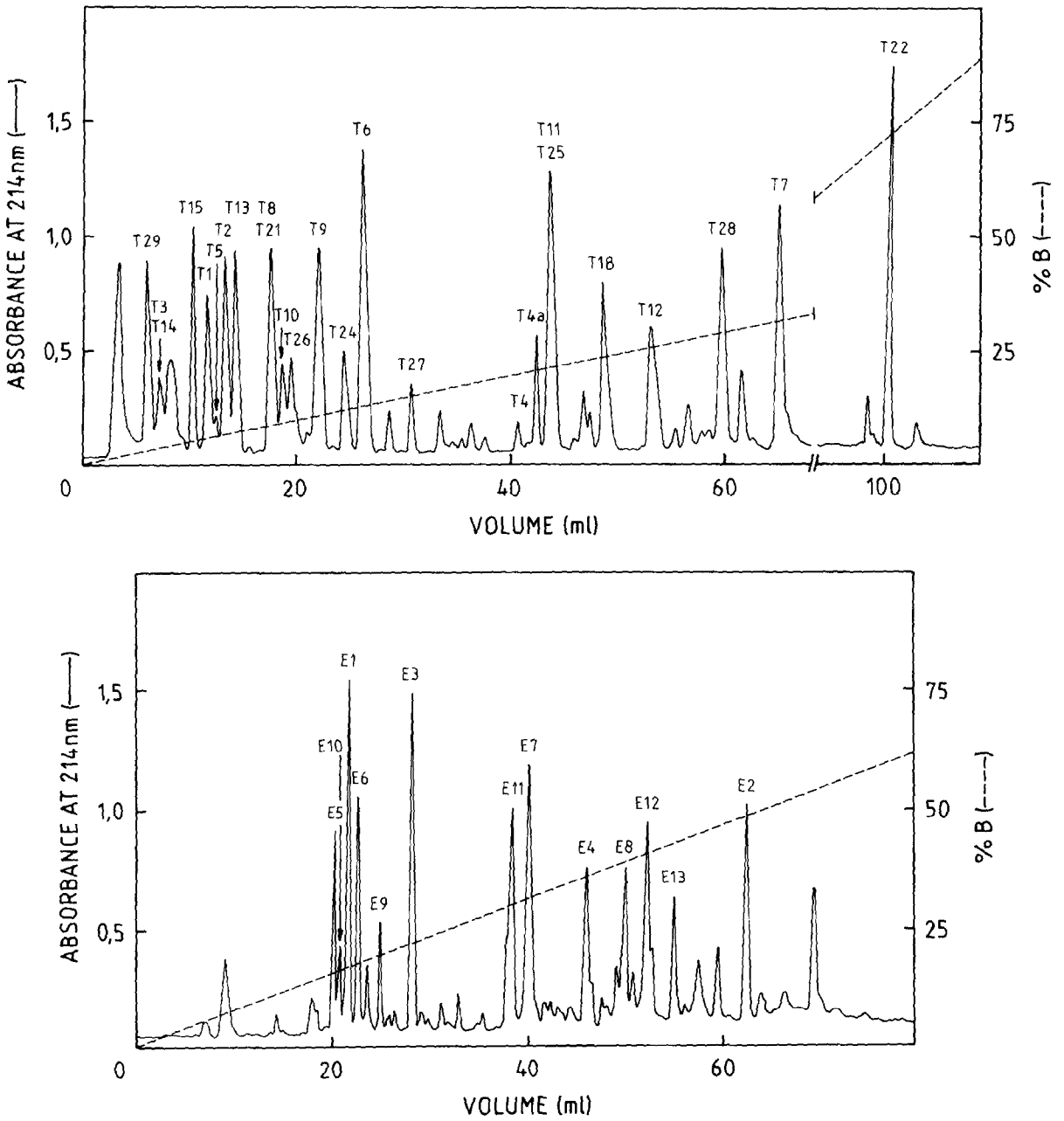

Fig.2. Purification by reverse-phase HPLC of peptides from HTH_α and HTH_β obtained by digestion of the mixture with trypsin (A) and with staphylococcal Glu-specific protease (B). The elution positions of all peptides are shown, using the same peptide nomenclature as in fig.1.

nor for N- or C-terminal proteolytic processings. The results therefore suggest that HTH preparations contain 2 polypeptide chains identical with histones 2A and 2B. The final structures deduced are compatible with results from SDS-polyacrylamide gel electrophoresis, with the total compositions (table 1), and with the fact that HTH preparations did not contain detectable amounts of other proteins.

About 5–15% of H2A and 1.5% of H2B have been reported to be linked to ubiquitin [13]. However, evidence was not obtained in the preparations analyzed here for the presence of peptides derived from proteins other than the 2 HTH molecules. It therefore appears as if the HTH preparations, although they contain histones, need not contain ubiquitin in sufficient quantities for detection in routine structural analysis. Naturally, it is still possible that ubiquitin is required for in vivo actions of the hormone, even if not detected in the HTH preparations.

The presently determined structures give full support for the suggestion of new histone functions. This suggestion was concluded from the extensive similarities between histones/HTH and other thymus hormones [6]. The conclusions also suggest that HTH may function via involvements in the regulation of protein metabolism, and that histones may have unexpected roles apart from those of being nucleosome components. Comparisons and correlations with histone/HTH structures and functions are further discussed in a separate study [6]. The present results show that those conclusions apply to preparations containing protein chains with complete identity to histone polypeptides.

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